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MUTAGENIC AND CARCINOGENIC POTENCY OF EXTRACTS OF DIESEL AND RELATED ENVIRONMENTAL EMISSIONS: SUMMARY AND DISCUSSION OF THE RESULTS

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The proposed conversion from gasoline powered automobiles to diesel powered vehicles has prompted the Environmental Protection Agency to evaluate the potential health effects associated with exposure to diesel emissions. At present, there is no direct epidemiological link between this exposure and human health. Therefore, a research program was constructed to compare the health effects associated with diesel emissions with those from other emission sources for which epidemiological information was available. The emission sources chosen were cigarette smoke, roofing tar, and coke oven. An additional comparative emission source which was evaluated was a gasoline catalyst engine. Respirable particles from a variety of combustion sources have the potential of being carcinogenic and mutagenic. The objective of these studies was to determine the relative biological activity of the organic material adsorbed on these particles in both *in vitro* mutagenesis and *in vitro* and *in vivo* carcinogenesis bioassays. The organic extracts from the following series of emission sources were quantitatively bioassayed in a matrix of tests for their carcinogenic and mutagenic activity: (1) a light-duty Oldsmobile diesel 350 engine; (2) a heavy-duty Caterpillar diesel engine; (3) a light-duty Nissan engine; (4) a Volkswagen Rabbit diesel engine; (5) cigarette smoke; (6) roofing tar; (7) coke oven; and (8) a gasoline catalyst Mustang. The test matrix consisted of the following bioassays: reverse mutation in *Salmonella typhimurium*; mitotic recombination in *Saccharomyces cerevisiae*; DNA damage in Syrian hamster embryo cells (SHE); sister chromatid exchange in CHO cells; gene mutation in L5178Y mouse lymphoma cells, Balb/c 3T3 mouse embryo fibroblasts and CHO cells; viral enhancement of SHE cells; oncogenic transformation in Balb/c 3T3 cells; and skin tumor initiation in SENCAR and C57 black mice. The results of this test matrix are discussed.

Introduction

The proposed conversion from gasoline powered automobiles to diesel powered vehicles has prompted the Environmental Protection Agency to evaluate the potential health effects associated with exposure to diesel emissions (Ember, 1979). At present, there is no direct epidemiological link between this exposure and human health (EPA, 1978). Therefore, a research program was constructed to compare the health effects associated with diesel emissions with those from other emission sources for which epidemiological information was available. The emission sources chosen were cigarette smoke, roofing tar, and coke oven. An additional comparative emission source which was evaluated was a gasoline catalyst vehicle.

Respirable particles from a variety of combustion sources have the potential of being carcinogenic and mutagenic (Waters *et al.*, 1979). The objective of these studies was to determine the relative biological activity of the organic material adsorbed on these particles in both *in vitro* and *in vivo* mutagenesis and carcinogenesis bioassays. The organic extracts from the following series of emission sources were quantitatively bioassayed in a matrix of tests for their carcinogenic and mutagenic activity: (1) a light-duty Oldsmobile diesel 350 engine, (2) a heavy-duty Caterpillar 3304 diesel engine, (3) a light-duty Datsun Nissan 220C engine, (4) a Volkswagen turbocharged Rabbit diesel engine, (5) cigarette smoke, (6) roofing tar, (7) coke oven, and (8) an unleaded gasoline catalyst Mustang II. The collection, characterization, and description of these samples are described by Lewtas *et al.* (1981).

The test matrix consisted of the following bioassays: reverse mutation in *Salmonella typhimurium*; mitotic recombination in *Saccharomyces cerevisiae*; DNA

breakage in Syrian hamster embryo cells (SHE); sister chromatid exchange in Chinese hamster ovary (CHO) cells; gene mutation in L5178Y mouse lymphoma cells, Balb/c 3T3 mouse embryo fibroblasts, and CHO cells; viral enhancement in SHE cells; oncogenic transformation in Balb/c 3T3 cells; and skin tumor initiation in SENCAR and C57 black mice.

The potency of complex mixtures of organic compounds in biological systems will depend on the uptake, distribution, metabolism, and binding of each component of the mixture in each biological system; the specific biological endpoint (e.g., gene mutation); the sensitivity of each biological system to the individual components of the mixture; the possible interactions, both synergistic and antagonistic, which arise from the exposure to multiple agents; and the type of quantitative method which is applied to the data. Differences in these parameters will result in altered potency.

Aware of these problems and shortcomings, we have attempted to correlate biological end effects from a variety of carcinogenesis and mutagenesis bioassays with the nature or source of a series of combustion samples. The purpose of this comparative study is to evaluate each of the *in vitro* bioassay systems in comparison with each other and with the *in vivo* system. This paper will summarize and discuss the results of the bioassays reported in the session on the Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions (Claxton, 1981; Mitchell *et al.*, 1981; Casto *et al.*, 1981; Curren *et al.*, 1981; Slaga *et al.*, 1981).

Results and Discussion

Quantitation method

General procedures. The method for relating effect with dose depends on the bioassay being considered. The bacterial mutation system of Ames (AMES), the sister chromatid exchange bioassay in Chinese hamster ovary cells (SCE), the mammalian cell mutagenesis bioassay in L5178Y mouse lymphoma cells, (L5178Y) and the mouse skin tumor initiation bioassay (TUMOR INITIAT) gave good dose-response relationships and the slope of the linear portion of the dose response curve was chosen for potency estimation (Table 1). The Balb/c 3T3 mutagenesis and oncogenic transformation bioassays (BALB) did not give good dose response with the samples tested and doses chosen. A quantitation method was therefore applied which utilized the lowest effective concentration tested (LECT) which exerted a biological response. It is to be emphasized, however, that with pure agents and more closely spaced doses the Balb assay does respond in a dose-related fashion. The viral enhancement bioassay in Syrian hamster embryo cells (VIRAL ENHANCE) gave dose related response information in terms of absolute frequency but for comparison to the Balb system and due to the kind of statistics applied to the assay, the LECT method was chosen. It is possible that the two methods, slope and LECT, when applied to the same data could give dissimilar relative rankings. Bioassay results in *Saccharomyces cerevisiae*, DNA damage in SHE cells, gene mutation in CHO cells, and skin tumor initiation in C57 black mice were not utilized in this analysis due to marginal results.

Table 1. Comparative potency: Quantitation method.

	Mutagenesis				Carcinogenesis		
Bioassays:	AMES	SCE	L5178Y	BALB	VIRAL ENHANCE	BALB	TUMOR INITIAT
Quantitation Method:	Slope	Slope	Slope	LECT	LECT	LECT	Slope

LECT = Lowest effective concentration tested.
Slope = Slope of the linear portion of the dose–response curve.

Normalization of data. In order to reduce or normalize the potencies in the bioassays to a common denominator, the following system was applied: the activity of the Nissan diesel sample regardless of bioassay type or quantitation method used was given a value of 100. All the results of other emission samples were then related to the Nissan sample. For example, in the Ames bioassay, Strain TA-98 less metabolic activation, the potency of Nissan and Oldsmobile by the slope method was 1225 and 615 revertants/100 μg, respectively. Assuming the Nissan potency equals 100 by simple mathematical relationship, the Oldsmobile sample equals 50 or one-half the potency of the Nissan sample. In the case of a LECT potency, the inverse relationship is applied. For example if the LECT for Nissan is 75 μg/ml and that for Cat is 300 μg/ml, assuming Nissan equals 100, then Cat equals $75/300 \times 100$ or 25, one-quarter the potency of the Nissan sample. Using this kind of analysis, the absolute data was converted to normalized values. The reason the Nissan was chosen as the sample to normalize to was that it was the only diesel sample tested in all bioassays which gave positive results.

Comparative rankings

Gene mutation assays. A comparison of the test results of the eight samples plus the positive control in the three gene mutation assays is found in Table 2. The three gene mutation assays compared were Ames strain TA-98, L5178Y at the $TK^{+/-}$ locus, and BALB 3T3 at the ATPase locus. Each system was performed without (−MA) and with (+MA) metabolic activation which consisted of an Aroclor-1254 induced rat hepatic S-9. The emission samples were previously described and are abbreviated as Cat for heavy duty Caterpillar; Nissan; Olds for Oldsmobile 350; VW Rabbit for the turbocharged diesel Rabbit; Mustang for the unleaded catalyst gasoline engine; cigarette for standard cigarette smoke condensate; coke for coke oven emissions; Roof

Table 2. Comparative rankings: Gene mutation assays.

Sample		AMES[a]		L-5178Y[b]		BALB/3T3[c]	
		−MA	+MA[d]	−MA	+MA	−MA	+MA
Diesel:	Cat	5.4	4.3	16	I[e]	75	25
	Nissan	100	100	100	100	100	100
	Olds	50	23	58	64	94	58
	VW Rab	33	22	21	50	I	I
Gasoline:	Mustang	11	25	32	36	300	7500
Comparative Sources:	Cigarette	0	7	42	21	300	300
	Coke	13	18	26	339	300	15
	Roof Tar	0	7	16	850	150	7500
Standards:							
	B(*a*)P	0	1112	0	189		25000
	MNNG					25000	

[a]*Salmonella typhimurium* strain TA-98.
[b]L-5178Y mouse lymphoma cells (TK$^{+/-}$ locus).
[c]BALB 3T3 mouse embryo fibroblasts (ATPase locus).
[d]Metabolic activation by an Aroclor-1254 induced rat hepatic S-9.
[e]Testing incomplete at this time.

Table 3. Comparative rankings: DNA damage assay.

Sample		SCE (CHO)[a]	
		−MA	+MA[b]
Diesel:	Cat	4	0
	Nissan	100	100
	Olds	0	0
	VW Rab	30	50
Gasoline:	Mustang	29	I[c]
Comparative Sources:	Cigarette	47	0
	Coke	171	44
	Roof Tar	60	291
Standards:	B(*a*)P	0	1750

[a]Sister chromatid exchange in Chinese hamster ovary cells.
[b]Metabolic activation by an Aroclor-1254 induced rat hepatic S-9.
[c]Testing incomplete at this time.

Tar for roofing tar emissions; B(*a*)P for benzo(*a*)pyrene; and MNNG for N-methyl-N′-nitro-N-nitrosoguanidine.

The Nissan, as per our definition, has a value of 100 in all assays. Both the Ames and lymphoma assay (without activation) show the same relative potency within the diesel samples with Nissan > Olds > Rabbit > Cat. When the gasoline sample is included, the rankings are: Ames: Nissan > Olds > Rabbit > Mustang > Cat; mouse lymphoma: Nissan > Olds > Mustang > Rabbit > Cat. Both of these cell types lack the oxidative enzymes required for the activation of mutagens or carcinogens. The diesel and gasoline samples show primarily direct acting activity. Balb 3T3 cells possess the enzymes required for the activation of carcinogens especially polycyclic aromatics (Curren *et al.*, 1981) and therefore one cannot compare all these systems across the board and expect similar results. Comparison of the three systems with metabolic activation showed the relative ranking (with some exceptions) to be: Nissan > Olds > Rabbit > Cat.

The comparative sources samples generally show more metabolic activation dependence for maximal effect and less quantitative correlation in the gene mutation assays.

DNA damage assay. The mobile source samples tested for sister chromatid exchange in CHO cells, without activation, (Table 3) gave the following ranking: Nissan > Rabbit, Mustang ≫ Cat, Olds.

In the presence of metabolic activation, the SCE results with the comparative sources ranked in similar order with the mouse lymphoma results: roof tar > coke > cigarette.

Oncogenic transformation. The results from assays which relate to the transformation of cells in culture are found in Table 4. Both of these systems were evaluated by the LECT method. The viral enhancement assay measures the ability of a chemical to enhance viral transformation in mammalian cells while the BALB assay measures the ability of the chemical to directly transform mammalian cells. SHE cells like BALB cells contain the enzymes needed for the metabolic activation of carcinogens especially polycyclic aromatics (Casto *et al.*, 1981). At this time not all the data points are complete in the Balb 3T3 assay. The mobile source samples, presently rank for viral enhancement in the absence of metabolic activation: Nissan > Rabbit, Mustang ≫ Cat, Olds, while the comparative samples rank: roof tar > coke > cigarette.

Mouse skin tumorigenesis. The results obtained in the SENCAR mouse skin tumorigenesis experiments for tumor initiators have been compared at 14 weeks after treatment (interim score) for all the samples and at 27

Table 4. Comparative rankings: Oncogenic transformation assays.

Sample		Viral Enhancement (SHE)	BALB 3T3	
			−MA	+MA[a]
Diesel:	Cat	0	1.3	0
	Nissan	100	100	100
	Olds	0	1.9	0
	VW Rab	50	NT[b]	NT
Gasoline:	Mustang	50	100	2000
Comparative Sources:	Cigarette	200	I[c]	I
	Coke	800	10	I
	Roof Tar	1040	I	500
Standards:	B(*a*)P	50000	—	16700
	MNNG	31250	8300	—

[a]Metabolic activation by an Aroclor-1254 induced rat hepatic S-9
[b]Not tested.
[c]Testing incomplete at this time.

Table 5. Comparative rankings: SENCAR mouse skin tumorigenesis.

Sample		Tumor Initiation		
		Interim Score[a]		Final Score
		Ranking	Potency[b]	Potency[b]
Diesel:	Cat	0	0.00	0.00
	Nissan	100	0.258	
	Olds	45	0.115	0.145
	VW Rab	I[c]	I	
Gasoline:	Mustang	35	0.090	
Comparative Sources:	Cigarette	0	0.00	
	Coke	119	0.307	
	Roof Tar	71	0.182	
Standards:	B(*a*)P	17900	46.2	71.6

[a] Interim score 14 weeks after treatment.
[b] Papillomas/mouse/mg.
[c] Testing incomplete at this time.

weeks of treatment (final score) for three samples (Table 5). The interim score is represented here in both absolute terms (papillomas/mouse/mg) and in the normalized rankings while the final scoring for comparison is in absolute terms. There are, as expected, differences in the slope potencies from incomplete vs completed experiments but those differences do not exceed 2-fold in the Olds and B(*a*)P samples. The Cat and cigarette samples were negative in this assay system up to 10,000 μg/mouse. The lack of activity of the cigarette smoke condensate may be due in part to the method of sample collection. This sample is not an organic extract of particles as are the other samples and is therefore comparatively much less concentrated. This may explain the very low percentage of benzo(*a*)pyrene found in the cigarette smoke condensate sample (Table 6). The Olds, Mustang, and roofing tar activities were less than Nissan while the coke oven sample was slightly higher. The ranking of the mobile source samples was: Nissan > Olds > Mustang ≫ Cat.

Table 6. Correlation between organics and tumor initiation.

		Percent Organic Extractable	ng B(*a*)P/mg Ext.	Mouse Skin Tumor Initiation[a]
Diesel:	Cat	27	2	0.00
	Nissan	8	1173	0.258
	Olds	17	2	0.115
	VW Rab	18	26	I[b]
Gasoline:	Mustang	43	103	0.09
Comparative Sources:	Cigarette	—	<1	0.00
	Coke	7	478	0.307
	Roof Tar	>99	889	0.182
Standards:	B(*a*)P	—	10[b]	46.2

[a] Interim score 14 weeks after treatment (papillomas/mouse/mg).
[b] Testing incomplete at this time.

A comparison of the amount of B(*a*)P per milligram extract and the potencies of those samples as skin tumor initiators is found in Table 6. There is little correlation observed, except for the Olds sample, between ng B(*a*)P/mg extract and papillomas/mouse/mg extract.

Comparison across test systems

The relative rankings of the mobile source samples are listed in Table 7. These results are from those bioassays performed in the presence of exogenous metabolic activation.

There is a consistency in these results with the Nissan sample the most potent and the Cat sample the least potent in all bioassays.

The relative rankings of the comparative source samples is found in Table 8. In three of five systems, the identical rank order of roof tar > coke > cigarette is found, and in all five systems, the cigarette sample was the least potent.

The normalized rankings for 7 bioassays are compared in Table 9. The results presented are from those bioassays performed in the presence of exogenous metabolic activation including Ames, SCE, L5178Y, and Balb-mutagenesis and transformation. The quantitative results from the mobile source samples show a general overall consistency. Cat, a very weak sample in Ames is inactive in SCE, viral enhancement, Balb transformation, and mouse skin tumor initiation. Olds, weak in Ames, L5178Y, Balb mutation, and mouse skin tumor initiation is inactive in SCE, viral enhancement, and BALB transformation. In three of the four assays for

Table 7. Relative rankings of mobile source samples.

Bioassay	Rank Order
AMES[a]	Nissan > Mustang, Olds, Rabbit ≫ Cat
L5178Y[a]	Nissan > Olds > Rabbit > Mustang
SCE[a]	Nissan > Rabbit ≫ Cat, Olds
Viral Enhancement	Nissan > Mustang, Rabbit ≫ Cat, Olds
Tumor Initiation[b]	Nissan > Olds > Mustang ≫ Cat

[a] In the presence of an Aroclor-1254 induced rat hepatic S-9.
[b] Mouse skin tumor initiation in SENCAR mice 14 weeks after treatment.

Table 8. Relative rankings of comparative source samples.

Bioassay	Rank Order
AMES[a]	Coke > Roof Tar, Cigarette
L5178Y[a]	Roof Tar > Coke > Cigarette
SCE[a]	Roof Tar > Coke ≫ Cigarette
Viral Enhancement	Roof Tar > Coke > Cigarette
Tumor Initiation[b]	Coke > Roof Tar ≫ Cigarette

[a] In the presence of an Aroclor-1254 induced rat hepatic S-9.
[b] Mouse skin tumor initiation in SENCAR mice 14 weeks after treatment.

Table 9. Comparative potency rankings.

		Mutagenesis				Carcinogenesis		
		AMES[a]	SCE[a]	L-5178Y[a]	BALB[a]	VIRAL ENHANCE	BALB[a]	TUMOR INITIAT[b]
Diesel:	Cat	4.3	0	I[c]	25	0	0	0
	Nissan	100	100	100	100	100	100	100
	Olds	23	0	64	58	0	0	45
	VW Rab	22	50	50	NT[d]	50	NT	I
Gasoline:	Mustang	25	I	36	7500	50	2000	35
Comparative Sources:	Cigarette	7	0	21	300	200	I	0
	Coke	18	44	339	15	800	I	119
	Roof Tar	7	291	850	7500	1040	500	71
Standards:	B(*a*)P	1112	1750	189	25,000	50,000	16,700	17,900

[a]In the presence of an Aroclor-1254 induced rat hepatic S-9.
[b]Mouse skin tumor initiation in SENCAR mice after 14 weeks of treatment.
[c]Testing incomplete at this time.
[d]Not tested.

which test data is available, SCE, L5178Y, and viral enhancement, the Rabbit sample gave almost identical quantitative results. The Mustang sample gave similar results in Ames, L5178Y, and mouse skin tumor initiation, while markedly dissimilar results were obtained in the Balb assay.

With the comparative source samples, there is little agreement between the quantitative results from these bioassays with the exception of the roofing tar sample in L5178Y, viral enhancement and Balb transformation.

In theory, gene mutation and skin tumor initiation arise from similar one hit, single process, irreversible mechanisms and should give similar results assuming equal toxicity and mutagen/carcinogen transport/activation by the various cell types. A comparison of the results of the mobile source samples in Ames and L5178Y gene mutation with those results in mouse skin tumor initiation seem to support this hypothesis.

In conclusion, a series of extracts from diesel and gasoline emission samples were evaluated in a battery of bioassays and a broad general agreement was found among most of the bioassays. Similar experimentation with the cigarette, coke oven, and roofing tar samples produced dissimilar results. Additional experimentation and analysis will continue in this important area of environmental mutagenesis and carcinogenesis.

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